EFFECTS OF ESTRADIOL AND PROGESTERONE ON RAT INTESTINAL AND HEPATIC PHOSPHOLIPASE A ACTIVITY

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The experiments reported herein were conducted according to the principles set forth in the current edition of the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

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Small intestine and liver play major roles in lipid and lipoprotein metabolism. Both organs contain high activities of phospholipase A, but little is known regarding the hormonal regulation of this enzyme in these tissues. In the present study, phospholipase A activity was measured in male rats treated with subcutaneous injections of 17 beta-estradiol or progesterone. Estradiol treatment (0.6 mg/day for 14 days) decreased intestinal and hepatic phospholipase A activities by 65% and 53%, respectively. Administration of estradiol reduced the specific activity of the enzyme in both microsomal and cytosol fractions of the intestinal mucosa. In contrast to the effects of estradiol, progesterone administration (2 mg/day for 14 days) increased phospholipase A activity in intestinal and hepatic tissue by 2.4- and 2.1-fold, respectively. The progesterone treatment raised the specific activity of phospholipase A in all subcellular fractions of both intestinal and hepatic tissue. These hormone-induced changes in phospholipase A activity may affect the regulation of plasma lipoprotein metabolism and the generation of prostaglandin in intestine and liver.

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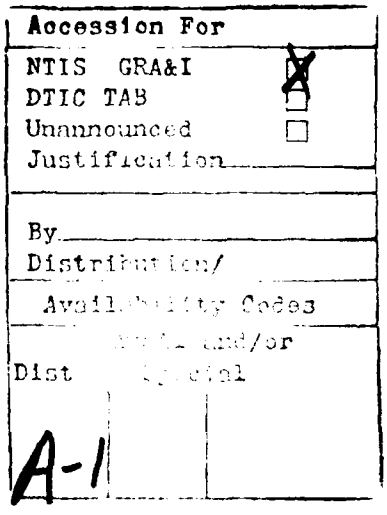

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INTRODUCTION

Phospholipase A is involved in the initial step in prostaglandin synthesis (1) and may also play a role in the metabolism of plasma lipoproteins (2). Several workers have investigated the effects of hormones upon phospholipase A activity. Rillema and Wild (3) showed that prolactin treatment increased phospholipase A activity in rat mammary gland membranes. In addition, Ehnholm et al. found that administration of the androgen oxandrolone raised phospholipase A activity in human postheparin plasma (4), and recently, Grieves and Liggins (5) reported increased phospholipase A activity of amnion and chorioallantois following stimulation of fetal adrenals with corticotropin. Liver and small intestine are key organs in lipid and lipoprotein metabolism, and both contain some of the highest activities of phospholipase A (6-8). Yet, little is known regarding hormonal regulation of this enzyme in these tissues. Therefore, the present investigation was undertaken to determine the effects of 17-Beta-estradiol and progesterone administration on phospholipase A activity in rat small intestine and liver.

MATERIALS AND METHODS

Materials. Progesterone and 17-Beta-estradiol (estradiol) were purchased from Research Plus Steroid Lab., Inc., Danville, New Jersey. The other materials used in this study were described previously (7,8).

Treatment of animals. Male Charles River rats, 6 to 7 weeks old, were fed Purina Laboratory Show, ad libitum, and had unlimited access to water. Two groups of animals received daily subcutaneous injections of estradiol (0.2 to 0.8 mg/day) or progesterone (1 to 3 mg/day) dispersed in 0.1 ml of propylene glycol. Control rats received 0.1 ml propylene glycol alone. Prior to sacrifice, all animals were anesthetized with sodium pentobarbital (6 mg/100 g) 24 hr after the last injection.

The amount of estradiol administered in the present study is much larger than the anovulatory doses used in rats (9-12). However, pharmacologic doses of estradiol have been used in several studies in the past (13). The doses of progesterone used are comparable to earlier investigations (9-11).

Preparation of Intestinal Tissue. After anesthetizing the animal, the entire small intestine was removed and divided into three equal segments. Only the distal intestinal segment was used in this study since previous work from this laboratory (7) had shown that this segment contained the highest phospholipase A activity. Subcellular fractions of distal intestinal mucosa were prepared as described previously (7,14).

Preparation of Hepatic Tissue. After removal, the liver was rinsed thoroughly with Tris buffer containing sucrose and magnesium chloride (0.05 M Tris, pH 7.4; 0.25 M sucrose; and 5 mM $MgCl_2$), dried on filter paper, and weighed. The liver was minced with scissors and then homogenized at 4°C with a motor driven Potter-Elvehjem homogenizer (10 strokes at 1300 rpm) using one volume of liver to two volumes of the above buffer. After filtration through four layers of cheese cloth, an aliquot of the homogenate was saved for phospholipase A assay and the remainder was further fractionated. The method of Berezney and Coffey (15) was used to prepare the nuclear fraction. Mitochondria, cytosol, and microsomes were prepared by the procedure of Hogeboom (16).

The purity of the various subcellular fractions of liver was confirmed by measuring the following marker enzymes: glucose-6-phosphatase and acid phosphatase (17), and cytochrome C oxidase (19). The distribution of glucose-6-phosphatase, an enzyme known to be associated with microsomes (17), was as follows: microsomes, 81.2%; cytosol, 8.5%; mitochondria, 6.2%; and nuclei

4.1%. The mitochondrial fraction contained 83.7% of the cytochrome oxidase activity. The microsomes, nuclei, and cytosol contained 7.7%, 5.6% and 3% of this enzyme, respectively. These results are in agreement with those of earlier studies (17). The mitochondrial fraction contained the highest percentage (79.7%) of acid phosphatase, a known lysosomal enzyme (19). This enzyme was also present in microsomes (10.9%), cytosol (5.8%), and nuclei (3.6%).

Assay of Phospholipase A Activity. Since administration of estradiol or progesterone may cause changes in the composition of hepatic or intestinal lipids, all assays were done on acetone powders (20) to remove fat from the tissues and subcellular fraction. Phospholipase A (EC 3.1.1.32 and 3.1.1.4, $A_1 = A_2$) activity was determined in these acetone powders by the method of Sundaram et al. (8), using dipalmitoyl ($^{1\text{-}14}C$) phosphatidylethanolamine as the substrate. Phospholipase A assay in acetone powders of intestinal mucosa was previously described in detail from this laboratory (7). Additional experiments have now characterized the enzyme assay in acetone powders of homogenates and subcellular fractions of liver. In these experiments, substrate concentration was not rate limiting at 4 umol/ml, the concentration used in the assay. The optimal pH of the hepatic enzyme was approximately 8.6. The rate of hydrolysis of phosphatidyl ethanolamine increased linearly with protein concentrations between 0.1 and 0.75 mg for the homogenate or nuclei, between 50 and 5400 ug for the microsomes, and between 0.5 mg and 2.5 mg for the mitochondria and cytosol. Enzymatic activity was linear for at least 90 min when the protein content of each fraction was within the ranges described above. In this paper, on unit of enzyme activity is defined as the release of 1 nmole palmitate h^{-1} at 37°C.

Previous studies demonstrated that Ca^{2+} activates hepatic phospholipase A_2 (21). However, addition of Ca^{2+} (1 to 10 mM) to the assay mixture did not alter phospholipase A activity in homongenates or subcellular fractions of liver.

Since rat liver contains phospholipase C (22), which hydrolyzes dipalmitoyl phosphatidylethanolamine to dipalmitin and phosphorylethanolamine, hydrolysis products were measured after incubation for various periods of time. Lipids were extracted from the incubation mixture and separated by thin-layer chromatography (23). Label in fatty acids accounted for 97% of the radioactivity recovered in assay products, and no label was found in mono- or diglycerides. These results show that hepatic homogenates exhibit no phospholipase C activity under these assay conditions and confirm that the observed hydrolysis of phosphatidyl ethanolamine was carried out by phospholipase A.

Determination of Protein. Protein determinations were performed by the method of Lowry (24).

Statistical Analysis. Statistical significance of the results was calculated by one way analysis of variance or by Newman-Keuls test (25) as applicable.

RESULTS

Dose Response Relationship Between Estradiol Treatment and Intestinal Phospholipase A Activity. As shown in Fig. 1, intestinal phospnolipase A activity in control animals did not change over the 21-day period examined in this study. In addition, treatment with 0.2 mg/day estradiol produced no significant change in enzyme activity. However, daily injections of 0.4 mg estradiol significantly ($p<0.05$) decreased the phospholipase A activity on days 14 and 21 of treatment. Daily administration of higher doses of estradiol (0.6 mg or 0.8 mg) further decreased phospholipase A activity on days 7, 14, and 21 of observation ($p<0.05$ vs control). For subsequent studies on the effects of estradiol on phospholipase A activity in subcellular fractions, we chose an estradiol dose of 0.6 mg/day for 14 days.

Dose Response Relationship Between Progesterone Treatment and Intestinal Phospholipase A Activity. Fig. 2 shows the effects of progesterone on intestinal phospholipase activity. Control rats showed no significant change in enzyme activity over the entire period of observation. Treatment with 1 mg/day progesterone did not significantly alter phospholipase A activity. However, daily administration of 2 or 3 mg of progesterone increased the activity of the enzyme on days 14 and 21 ($p<0.05$). For subsequent investigations involving the subcellular distribution of the enzyme rats were treated with 2 mg progesterone daily and were sacrificed on the fourteenth day.

Figure 1

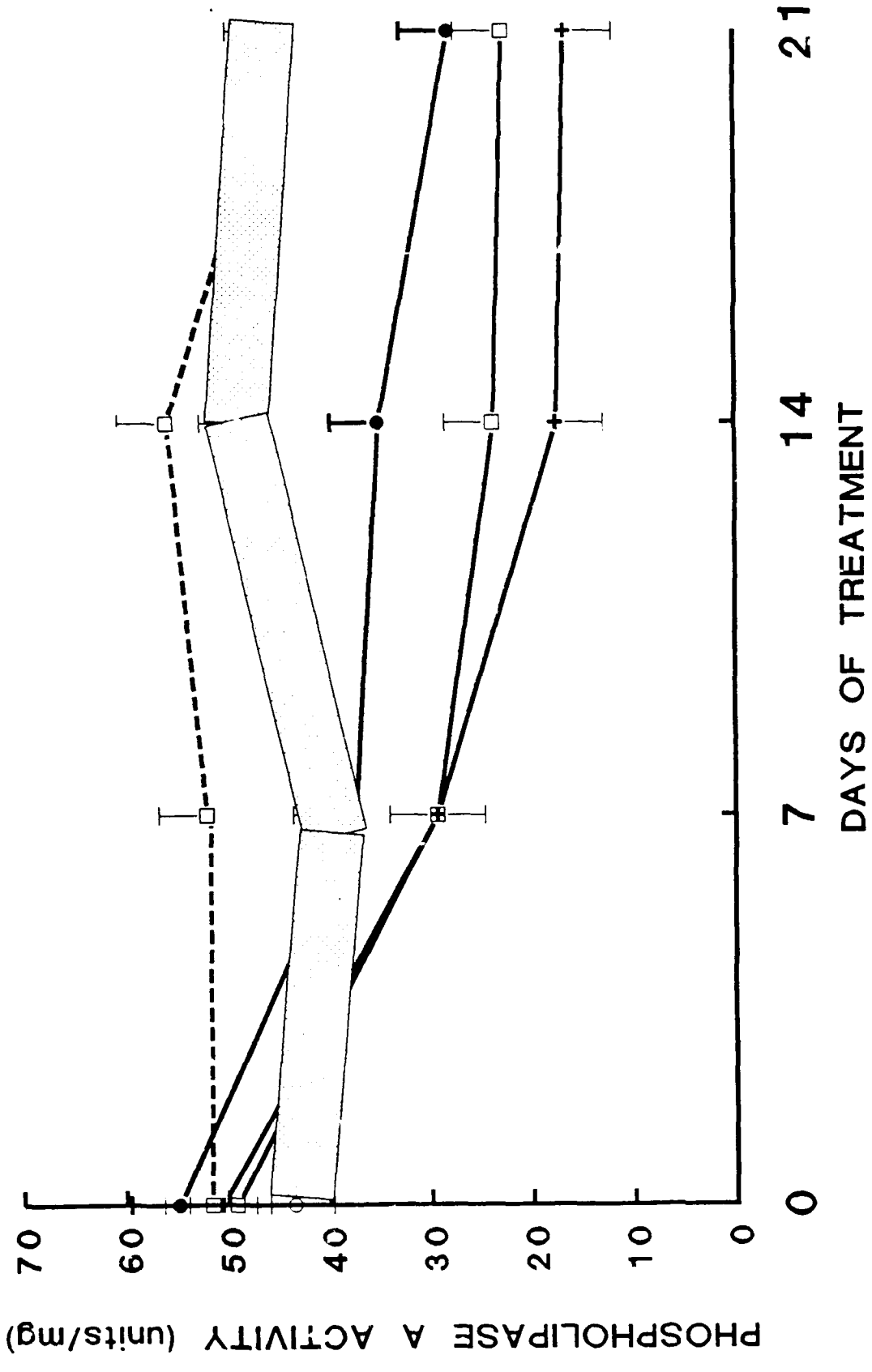
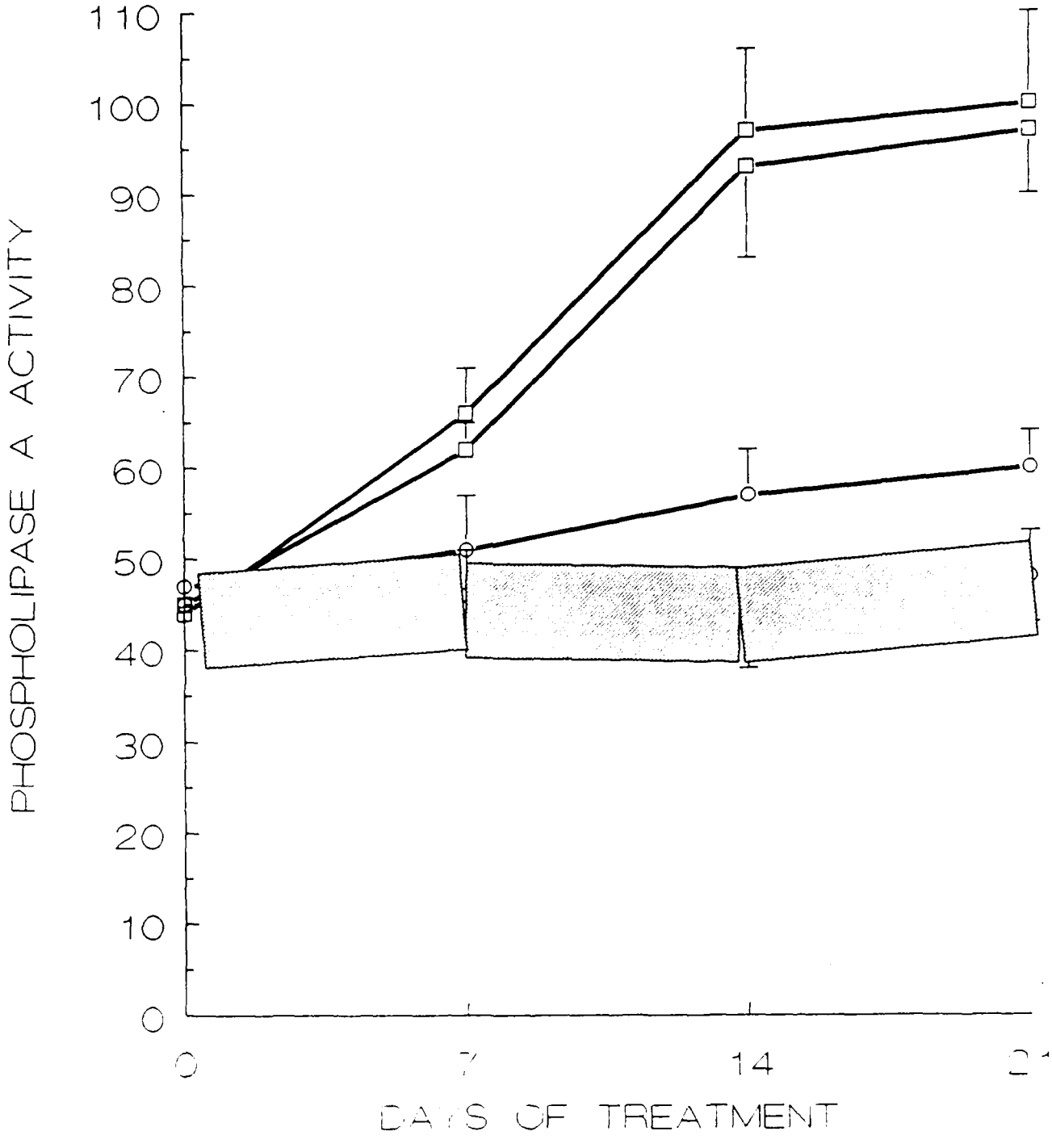

PHOSPHOLIPASE A ACTIVITY (units/mg)
70
60
50
40
30
20
10
0
0
7
14
21
DAYS OF TREATMENT

Figure 2


110
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PHOSPHOLIPASE A ACTIVITY
0
7
14
DAYS OF TREATMENT

Effects of Estradiol and Progesterone Treatment on Phospholipase A Activity of Subcellular Fractions of Intestine. The data in Table 1 show that the nuclei and brush border fraction contained the largest percentage of phospholipase A activity (40%) in the distal intestinal mucosa of control animals. The enzyme activity in the other fractions was as follows: mitochondria, 33% microsomes, 16%; and cytosol, 5%. Recovery of activity from the combined subcellular fractions was 94%. In estradiol-treated rats, the total enzyme activity decreased significantly ($p<0.0005$) from 82 x 10^3 to 29 x 10^3 units. However, the relative contribution of each subcellular fraction did not change when compared to control.

With progesterone administration, phospholipase A activity in distal intestinal mucosa increased from 82 x 10^3 to 200 x 10^3 units ($p<0.0005$). In addition, the data showed a significantly higher ($p<0.0005$) percentage of enzyme activity in the nuclei-brush border fraction from progesterone-treated rats (51% compared with 40% in controls). Correspondingly, there was a significantly lower ($p<0.0005$) percentage of phospholipase A activity in the microsomal and cytosolic fractions of the progesterone-treated group.

Effects of Hormone Treatment on the Specific Activity of Intestinal Phospholipase A. Table 2 shows that the specific activity of phospholipase A was highest in the microsomal fraction (126 units/mg protein), followed by mitochondria (84 units/mg), crude nuclei-brush border (54 units/mg), and cytosol (4.7 units/mg). The specific activity of the enzyme declined in all

TABLE 1

Effects of Estradiol and Progesterone on Phospholipase A_2 Activity in Homogenates and Subcellular Fractions of Rat Distal Intestinal Mucosa

Treatment Group	Enzyme activity in whole homogenate*	% Enzyme Activity in Subcellular Fractions				Enzyme Recovery %
		nuclei & brush border	mitochondria	microsomes	cytosol	
Control	82.3 ± 6.4	39.9 ± 3.7	32.8 ± 1.9	16.3 ± 1.2	5.12 ± 0.74	94.1
Estradiol	28.6# ± 4.7	33.7 ± 4.1	35.6 ± 1.7	18.0 ± 1.9	3.53 ± 0.63	90.8
Progest-erone	200# ±25	51.4# ± 2.0	34.8 ± 1.8	8.11# ± 0.72	2.64# ± 0.51	96.9

Phospholipase A activity was determined in acetone powders of homogenates and subcellular fractions as described in Methods. Rats received daily injections of either 0.6 mg estradiol, 2.0 mg progesterone, or vehicle for 14 days.

*Total enzyme activity is expressed as units 10^3.

Values represent the mean ± SEM, n = 6 animals per group. Statistical analyses were done by analysis of variance.

#Significantly different from control, $P<0.0005$.

TABLE 2

Effects of Estradiol and Progesterone on the Specific Activity of Phospholipase A in Homogenate and Subcellular Fractions of Rat Intestinal Mucosa

Subcellular Fraction	Control		Treatment Group			
			Estradiol		Progesterone	
	specific activity*	relative specific activity	specific activity	relative specific activity	specific activity	relative specific activity
Homogenate	43.7 ± 8.0	35	20.3 ± 5.4§	26	94.8 ± 12.5	56
Nuclei & brush border	54.4 ± 5.6	43	43.1 ± 7.2	55	67.4 ± 5.9#	40
Mitochondria	84.1 ±12.	67	80.7 ± 9.5	103	181 ±17.§	103
Microsomes	126 ±15.2	100	78.2 ± 8.9§	100	171 ±15.§	100
Cytosol	4.71 ±0.82	3.8	1.11 ±0.42§	1.4	9.73 ±1.5§	5.7

Phospholipase A activity was determined in acetone powders of homogenate and in subcellular fractions as described in Methods. Rats received daily treatment with either 0.6 mg estradiol, 2.0 mg progesterone, or vehicle for days.

*Specific activity data are expressed as units . mg protein^{-1}. Statistical analysis was done by analysis of variance.

#Significantly different from control, $P<0.01$

§Significantly different from control, $P<0.0005$

subcellular fractions during estradiol treatment. However, the fall was statistically significant only in the whole homogenate, microsomes, and cytosolic fraction ($p<0.0005$). The relative specific activity of phospholipase A decreased in the homogenate and cytosol and increased in nuclei-brush border and mitochondrial fractions.

In progesterone-treated rats, the specific activity increased significantly in all subcellular fractions. In this group the relative specific activity of the enzyme was higher in homogenate, mitochondria, and cytosolic fractions, whereas the relative specific activity fell slightly in the nuclei-brush border fraction.

Effects of Estradiol and Progesterone Treatment on Phospholipase A Activity in Homogenates and Subcellular Fractions from Liver. Table 3 shows that phospholipase A activity highest in the mitochondrial fraction (36%), followed by cytosol (32%), microsomes (14%), and nuclei (8.6%). Recovery of activity from the combined subcellular fractions was 91%. With estradiol treatment, the total enzyme activity in the rat liver homogenate decreased significantly from 120×10^3 units to 52×10^3 units ($p<0.01$). The relative contribution of each subcellular fraction to the total phospholipase A activity was essentially the same in estradiol-treated and control rats.

In progesterone-treated rats, the total phospholipase A in the liver increased significantly from 120×10^3 to 250×10^3 units ($p<0.0005$). The relative contribution of each subcellular fraction to the total phospholipase A activity remained the same in the progesterone-treated group as in the control.

TABLE 3

Effects of Estradiol and Progesterone Treatment on Phospholipase A in Homogenates and Subcellular Fractions of Rat Liver

Treatment Group	Enzyme activity in whole homogenate*	% Enzyme Activity in Subcellular Fractions				Enzyme Recovery %
		nuclei	mitochondria	micorosomes	cytosol	
Control	120 ±17	8.63 ± 2.5	36.4 ± 5.7	14.1 ± 1.5	31.5 ± 3.1	90.6
Estradiol	52 ±13#	9.32 ±1.3	41.3 ± 3.01	15.4 ± 1.7	28.7 ± 3.2	94.7
Progest-erone	250§ ±27	8.14 ± 1.2	39.8 ± 2.6	16.3 ± 1.8	29.9 ± 2.5	94.1

Phospholipase A activity was determined in acetone powders of homogenate and in subcellular fractions as described in Methods. Rats received daily treatment with either 0.6 mg estradiol, 2.0 mg progesterone, or vehicle for 14 days.

Total enzyme activity is expressed as units . 10^3. Values represent the mean ± SEM, n = 6 animals per group. Statistical analysis was done by analysis of variance.

#Statistically different from control, $P<0.01$

§Statistically different from control, $P<0.0005$

Effects of Hormone Treatment on the Specific Activity of Hepatic Phospholipase A. Table 4 shows the specific activity of phospholipase A in various subcellular fractions of rat liver. In control rats, the microsomes contained the highest specific activity (40 units/mg protein), followed by nuclei (11 units/mg), cytosol (5.8 units/mg), and mitochondrial fraction (2.4 units/mg). The specific activity of the enzyme decrease in the homogenate and in all subcellular fractions with estradiol treatment, but the values were significantly less than the controls only in the homogenate ($p<0.05$).

Table 4 also shows that the homogenate and each subcellular fraction from progesterone-treated rats. In this group of progesterone-treated rats, the relative specific activity of phospholipase A increased in the nuclei and mitochondrial fractions, whereas the relative specific activity was unchanged in the cytosolic fraction.

Weight gain during hormone treatment. Because steroid treatment is known to alter weight gain and this may effect the interpretation of our results, we measured the weights of all animals throughout the period of investigation. The data in Table 5 show that the initial weight (day 0) were similar in all groups. Each group of rats gained weight during the 21 days of the study; however, daily treatment with each dose of either estradiol or progesterone retarded the weight gain. In most treatment groups, changes were significantly different from controls early as the seventh day, and continued throughout the 21-day study. Most of the effects of estradiol or progesterone on weight gain were highly significant ($p<0.0005$).

TABLE 4

Effects of Estradiol and Progesterone on the Specific Activity of Phospholipase A in Homogenate and Subcellular Fractions of Rat Liver

Subcellular Fraction	Control		Treatment Group			
			Estradiol		Progesterone	
	specific activity*	relative specific activity	specific activity	relative specific activity	specific activity	relative specific activity
Homogenate	16.7 ± 5.0	42	10.0 ± 4.5#	50	39.5 ± 4.6¶	43
Nuclei	10.8 ± 4.7	27	5.53 ± 3.5	19	59.2 ±21.‖	65
Mitochondria	2.42 ±1.3	6	2.02 ± 0.91	7	10.0 ± 3.2‖	11
Microsomes	39.7 ±13.	100	28.3 ±15.	100	91.1 ±21.‖	100
Cytosol	5.81 ± 2.8	15	3.13 ±1.7	11	13.9 ± 4.9§	15

Phospholipase A activity was determined in acetone powders of homogenate and in subcellular fractions as described in Methods. Rats received daily treatment with either 0.6 mg estradiol, 2.0 mg progesterone, or vehicle for 14 days.

*Specific enzyme activity is expressed as units . mg protein^{-1}. Values represent the mean ± SEM, n = 6 animals per group. Statistical analysis was done by analysis of variance.

#P<0.05

¶P<0.025

‖P<0.005

¶P<0.0005

TABLE 5

Rat Weight During Treatment with Estradiol or Progesterone*

Treatment, dose	Days of Treatment			
Estradiol, mg	0	7	14	21
I - 0	175±5.6	218±11	254±11	289±12
II - 0.2	161±10.1	187± 8.9§	212±13§	238± 8.3§
III - 0.4	170± 5.5	192± 5.4§	218±11§	230± 8.3§
IV - 0.6	163± 9.3§	188± 9.3§	204±12§	208±15§
V - 0.8	185±4.7	205± 7.7	210±13§	218± 9.9§
Progesterone, mg				
I - 0	168± 3.8	225± 9.7	276± 9.6	314± 6.1
II - 1.0	173± 5.7	203±11#	239±14§	259±17§
III - 2.0	164± 3.9	209±10§	232±13§	241±12§
IV - 3.0	160± 4.7	198± 8.8§	213± 9.6§	225±11§

*Values represent the means ± SEM of 8 rats expressed in grams. Statistical analysis was done by analysis of variance.

#Significantly different from control rats on the corresponding treatment day, $P<0.025$

§Significantly different from control rats on the corresponding treatment day, $P<0.0005$

DISCUSSION

Investigations of the effects of sex hormones on phospholipase A activity are limited. However, one study by Ehnholm et al. (4) demonstrated that the anabolic steroid oxandrolone increased the activity of phospholipase A in postheparin plasma. In the present study, estradiol treatment decreased phospholipase A activity in liver and intestine, whereas progesterone administration increased enzyme activity in both tissues. Thus, estradiol reduces hepatic and intestinal phospholipase A activity, as well as the activity, as well as the activity of adipose tissue lipoprotein lipase (9-11). However, unlike its effects on other lipase (9-11), progesterone markedly increased phospholipase A activity in the liver and small bowel. The exact significance of these changes in phospholipase A activity on lipid metabolism is not clear. However, sex hormone administration has been shown to alter the apoprotein composition of plasma lipoproteins (12). These changes are thought to be due to the effects of sex hormones on the liver (12). The changes in phospholipase A activity in liver and intestine induced by sex hormone treatment may result in altered metabolism of the phospholipid fraction of plasma lipoproteins.

The nutritional status of the rats during hormonal treatment is a crucial issue. The reduction in weight gain with estrogen administration was more pronounced than in the progesterone-treated rats. However, the animals appears healthy and none of the rats died during the entire period of observation. Several lines of evidence suggest that the diminished weight gain alone cannot account for the changes in phospholipase A activity. Although the decreases in weight gain in all groups of estradiol-treated rats were similar, significant reductions in enzyme activity were observed only

with doses of 0.4 mg or higher. In addition, our previous studies showed that intestinal phospholipase A activity increased with fasting (7). Furthermore, both hormones retarded weight gains, but progesterone increased, while estradiol decreased phospholipase A activity. These data, taken together, imply that the hormone administration did not alter tissue phospholipase A activity through changes in nutritional status alone.

The relative contribution of the various subcellular fractions to the highest contribution was in the nuclei and brush border fraction, followed by mitochondria, microsomes, and cytosol. In the liver, the highest percentage of enzyme activity was in mitochondria, followed by cytosol, microsomes, and nuclei. Using a different separation procedure, several groups have demonstrated phospholipase A activity in both mitochondria and microsomes from rat liver (26). However, in these studies, detailed attention was not paid to the distribution of the enzyme in a purified nuclear preparation, and this may explain the discrepancy between the present study and the earlier ones.

Estradiol administration did not changes the percentage distribution of total phospholipase A activity in various subcellular fractions from intestinal mucosa or liver. However, in progesterone-treated rats the contribution by nuclei and brush border in intestinal mucosa was significantly higher, whereas that of the microsomal fraction was significantly lower. The highest specific activity of phospholipase A was found in the microsomal fraction in both liver and intestinal tissue. Treatment with estradiol decreased the specific activity of the enzyme in the microsomal and cytosol fraction of the intestinal mucosa, while progesterone treatment increased the specific activity of all the hepatic subcellular fractions. These alterations caused by pharmacological doses of estradiol and progesterone probably result

from many factors that effect turnover of specific proteins in the subcellular fractions of various tissues.

Despite the fact that the intestine is a major organ involved in lipid and lipoprotein synthesis, few studies have examine the effects of hormones on lipid metabolism in the small intestine. Furthermore, it has been suggested that hormone-induced alteration in lipoprotein lipase and hepatic triglyceride lipase activities may play a role in estrogen-induced hypertriglyceridemia (9-13). No studies have examined the effects of sex hormone administration on lipid metabolism in this organ. In fact, this is the first description of a response in the activity of a lipase in small intestine to the administration of sex hormones. Phospholipase A, an enzyme involved in providing arachidonic acid, is considered to play a role in prostaglandin synthesis (1). Recent studies have shown that both estrogens and testosterone regulate prostaglandin production in aorta, thrombocytes, and vas deferens (27,28). Whether sex hormones change prostaglandin synthesis in liver and small intestine remains to be investigated. Furthermore, these changes in phospholipase A activity may also play a part in altered lipoprotein metabolism induced by these hormones.

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FIGURE LEGENDS

Figure. 1. Dose response relationship between estradiol treatment and phospholipase A activity in intestinal mucosa. Rats were treated with daily injections of either vehicle, O--O or estradiol, 0.2 mg, ▪--▪; 0.4 mg, O--O; 0.6 mg, ▪--▪; or 0.8 mg x--x. Rats were sacrificed at various times and the intestinal mucosa was analyzed for phospholipase A activity, as described in the methods. Values represent the mean ± SEM, n=6 animals per group. Statistical analysis was done by the Newman-Keuls Test (25). *Significantly different from control, $p<0.05$.

Figure. 2. Dose response relationship between progesterone treatment and phospholipase A activity in intestinal mucosa. Rats were treated with daily injections of either vehicle, O--O or progesterone; 1.0 mg, O--O; 2.0 mg, ▪--▪; or 3.0 mg, ▪--▪. Rats were sacrificed at various times and the intestinal mucosa was analyzed for phospholipase A activity as described in the methods. Values represent the mean ± SEM, n = 6 animals per group. Statistical analysis was done by Newman-Keuls Test (25). *Significantly different from control $p<0.05$.